were also used to compare the raft affinity of wildtype and mutated PFO in vesicles containing ordered domains. Preliminary studies indicate in cholesterol-containing vesicles having wide Lo domains (distearoylphosphatidylcholine) and thin Ld domains (dimyristoleoylphosphatidylcholine), l-PFO showed higher raft affinity, whereas s-PFO shows little raft affinity. The raft affinity of l-PFO and s-PFO was reversed in vesicles containing thinner Lo domains (dipalmitoylphosphatidylcholine/dimyristoylphosphatidylcholine) and thick Ld domains (dieicosenoylphosphatidylcholine). These studies suggest that hydrophobic match may have an important role in TM protein raft affinity.

1512-Pos Board B282

Assessment of Membrane Deformation Continuum Elastic Models Based on Molecular Simulations of Gramicidin A

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We have assessed the continuum elastic model (CEM) for membrane deformations by comparison with all-atom molecular dynamics (MD) simulation results of gramicidin A (gA) dimers inserted into four different lipid bilayers formed by DLPC, DMPC, DOPC, and POPC. The CEM based on the smectic liquid crystal assumption for lipid membrane has been reported to efficiently model the protein-induced membrane deformation. In this description, the hydrophobic mismatch is assumed to be the dominant non-specific physical interaction between protein and lipid bilayer, and the deformation energy is modeled by compression-expansion, splay-distortion, and surface tension contributions. Using various boundary conditions (constrained, relaxed, and MD-based) and elastic modulus profiles (uniform and space-dependent), the deformation profile and the resulting energy were calculated and compared with the MD simulation results. Good agreement of the CEM and MD results is obtained for bilayer thickness profiles beyond the first lipid shell region from the gA channel when the MD-based boundary condition is used at lipid-protein contact. However, the continuum membrane representation near the protein contact, in the first shell, does not capture the atomic-level protein-lipid interactions. Thus careful/separate treatment of the first lipid shell region is required for the CEM to fully represent the realistic lipid bilayer deformation, as deduced from MD simulations. Introducing a space-dependent modulus profile has little effect on the deformation profile, but the calculated deformation energy depends strongly on the modulus profile.

1513-Pos Board B283

Relating Molecular Interactions with N-BAR Domains to the Mesoscopic Nature of Membrane Remodeling

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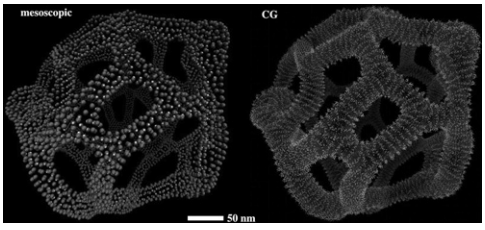
Theoretical approaches to studying biological problems have seen appreciable advancements in the past decade, allowing detailed structural and thermodynamic description of fundamental cellular processes, such as protein folding, lipid self-assembly, large-scale structural rearrangements of macromolecules, etc. Nevertheless, a full atomic description of even simple microscopic biological systems requires billions of atoms, beyond the reach of current computational methods. Membrane remodeling induced by members of the BAR domain protein family, is an innately multiscale problem, in which molecular interactions between the BAR protein and the lipids induces local curvature, and ultimately leads to large-scale reticulations of liposomes. In our study, we start with a discretized field-theoretical description of a liposome and carry out continuum mechanics simulations that generate reticulated topologies, quite similar to the ones seen in experiments. The obtained configurations are used as templates for mapping coarse-grained lipids and BARs over the continuum model. Subsequently, we use the coarse-grained representation of the liposome to run massive molecular dynamics simulations, with the aim of capturing the underlying molecular mechanisms that direct the liposome to undergo such large-scale biological restructuring and detecting the preferred localization of BARs at high resolution.



1514-Pos Board B284

Connecting Model Membrane Experiments to In Vivo Studies: DHA Acyl Chains Incorporate into Raft-Like Membranes more than EPA in Model Membranes, In Vitro, and In Vivo

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N-3 polyunsaturated fatty acids (PUFA) enriched in fish oil are increasingly recognized to have potential benefits for treatment of inflammatory and metabolic disorders; however, their mode of action remains unclear. One emerging hypothesis is n-3 PUFA acyl chains disrupt the molecular organization of lipid raft domains. In the present study, we first compared the effects of the two major n-3 PUFAs of fish oil, eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids, on molecular organization in model membranes. Solid state NMR spectroscopy revealed that more DHA- than EPA-containing phosphatidylcholine (PC) infiltrated into sphingolipid/cholesterol enriched raft-like domains. The tendency for DHA to incorporate into rafts was more than twice that for EPA. We then tested our results from model membranes in cell culture and in animals. In vitro, treatment of EL4 cells with EPA and DHA showed greater incorporation of DHA than EPA acyl chains into raft-like detergent resistant membranes (DRMs). Ex vivo, feeding C57BL/6 mice a diet enriched in EPA and DHA also resulted in a greater incorporation of DHA than EPA in PCs of DRMs of B cells. Total internal reflection fluorescence imaging confirmed the in vivo biochemical studies to show that fish oil dramatically disrupted the clustering of lipid raft domains. Taken together, our data demonstrate that model membrane studies can be effectively integrated with studies in cells and animals; moreover, we establish a potential mechanism by which EPA and DHA can differentially disrupt lipid raft molecular organization, which has implications for developing fish oil as a therapeutic in the clinic.

1515-Pos Board B285

Sub-Resolution Lipid Domains in the Plasma Membrane Influence Diffusion and Clustering

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Ordered membrane lipid microdomains have been postulated to regulate protein signaling by influencing the diffusion and clustering properties of membrane proteins. However, direct observation of lipid domains in cell membrane has been difficult. Here, we used fluorescence lifetime imaging of the order-sensitive dye Laurdan and phasor analysis to show the plasma membrane comprises a sub-resolution mixture of ordered and disordered domains. Simultaneous measurements of membrane order and protein diffusion showed that the membrane protein LAT (Linker for Activation of T cells) transiently interacted with sub-resolution domains, which retarded its diffusion. Palmitoylation-deficient LAT (C-S LAT) did not interact with lipid domains. In contrast, depolymerization of cortical actin had no effect on the abundance of lipid domains but directly affected the diffusion of LAT. Using super-resolution photo-activated localization microscopy (PALM) and quantitative cluster analysis, we demonstrated that diffusion correlated with LAT clustering. Hence treatments that modulate the abundance of sub-resolution lipid domains indirectly affect LAT clustering by altering diffusion, while cytoskeletal fences have a direct effect on the protein behavior. In conclusion, our data provide first insights into the molecular organization of the plasma membrane and how its biophysical properties may be exploited in the assembly of protein complexes.

1516-Pos Board B286

Partitioning, Diffusion, and Ligand Binding of Raft Lipid Analogs in Model and Cellular Plasma Membranes

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Several simplified membrane models featuring coexisting liquid disordered (Ld) and ordered (Lo) lipid phases have been developed to mimic heterogeneous organization of cellular membranes, for example into ordered lipid-protein nanodomains, termed ‘‘rafts’’. In spite of their greatly reduced complexity, quantitative characterization of local lipid environments using model membranes is not trivial, and the parallels that can be drawn to cellular membranes are not always evident. Similarly, various fluorescently labeled lipid analogs have been used to study membrane organization and function in vitro, while the biological function of these probes in relation to their native

counterparts remains unknown, especially of lipids such as sphingolipids that, when unlabeled, enter ''raft'' domains in model systems, termed ''raft'' lipids. Here, we analyze the phase partitioning of a multitude of fluorescent ''raft'' lipid analogs in synthetic Giant Unilamellar Vesicles (GUVs) and cell-derived Giant Plasma Membrane Vesicles (GPMVs). We observe complex partitioning behavior dependent on label size, polarity, charge and position, lipid headgroup, and membrane composition. Several ''raft'' lipid analogs partitioned into the ordered phase in GPMVs, in contrast to fully synthetic GUVs, in which most ''raft'' analogs mis-partitioned to the disordered phase. This behavior correlates with the greatly enhanced order difference between coexisting phases in the synthetic system. In addition, not only partitioning but also ligand binding of the lipids is perturbed upon labeling: while cholera toxin B binds unlabeled GM1 in the Lo phase, fluorescently labeled GM1 binds exclusively in the Ld phase. Fluorescence correlation spectroscopy (FCS) by stimulated emission depletion (STED) nanoscopy on intact cellular plasma membranes consistently reveals a constant level of confined diffusion for ''raft'' lipid analogs which vary greatly in their partitioning behavior, suggesting different physicochemical bases for these phenomena.

1517-Pos Board B287

bim-FCS Analysis of Membrane Protein Diffusion Reveals Dynamics of Membrane Cytoskeleton and Lipid Domains in Intact Cells

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Signaling transmembrane proteins are experiencing a heterogeneous structure due to membrane cytoskeleton meshwork and selective association with cholesterol stabilized lipid domains throughout the cell membrane. The limited optical resolution due to diffraction limits direct observation of both cytoskeleton structure and cholesterol stabilized micro-domains.

Fluorescence correlation spectroscopy (FCS) allows to measure membrane protein diffusion, but does not resolve the heterogeneity. We analyze the diffusion of GFP-tagged membrane proteins in multiple areas of increasing size simultaneous using TIRF illumination and camera based FCS. The binned-imaging FCS (bimFCS) allows distinguishing free Brownian diffusing proteins from proteins interacting with the membrane cytoskeleton and from proteins transiently entering sub-microscopic domains of reduced mobility. Using bimFCS it is possible to study how subtle changes modulate the structural nanodomains or the functional interaction with them, and how these changes propagate with time.

We investigate effect of membrane meshwork on diffusion of transmembrane proteins and on GPI anchored proteins which associate with cholesterol stabilized micro-domains in intact cells. We show in real-time how the protein-membrane ultrastructure interactions are modulated by protein dimerization or lipid cross-linking. We perform Monte Carlo simulations of bimFCS experiments with square pixels to analyze the effect of cytoskeleton barriers and cholesterol domains on the diffusion. We are able to quantitatively model the changes induced by protein or lipid cross-linking.

1518-Pos Board B288

Direct Observation of Junctional Microdomain Assembly

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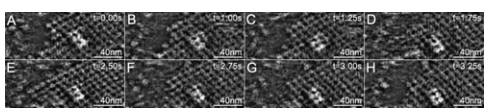
Junctional microdomains are specialized membrane protein assemblies in eye lens fiber cell membranes. They are constituted of lens-specific aquaporin-0 (AQP0) and connexins (Cx) that assure nutrition and adhesion of lens cells, mutation or absence of these proteins lead to cataract. In a more general term, junctional microdomains are paradigm for membrane protein segregation in functional assemblies in a membrane with non-random superstructure. Here we use high-speed atomic force microscopy (HS-AFM) and Monte Carlo simulation to analyze the dynamics and assembly of membrane proteins in junctional microdomains. We report cooperative adhesion of head-to-head attached AQP0 and Cx in junctional microdomains. Furthermore we evidence that enthalpy energy gain of protein association dominates entropy leading to square shaped AQP0 arrays of finite size segregated from and edged by connexins. The power of HS-AFM sample manipulation and imaging structure and dynamics at single-molecule resolution is highlighted opening a new avenue of membrane research watching the behavior of unlabelled molecules while also seeing their molecular environment.

HS-AFM movie frames showing native AQP0 arrays assembling (A-E) and disassembling (E-H).



1519-Pos Board B289

Local Cell Membrane Stiffness and Viscosity Mapped by Thermal Noise Imaging

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Cell membrane signaling depending on lateral diffusion is modulated by sub-microscopic membrane domains, such as cholesterol stabilized domains and membrane cytoskeleton fences. Thermal noise imaging (TNI) uses and optical trap to track the diffusion of a colloid labeled membrane protein with microsecond and nanometer. We show here how this method can be used to resolve the local membrane viscosity, accessibility and membrane stiffness with a few nanometer resolution in intact living cells. This require linearization of the detection and use of an optical trap to confine the diffusion to a small area of the membrane providing sufficient statistics for high resolutions maps of the diffusion. We find areas of reduced diffusion which are cholesterol dependent. These area are also stiffer than the remaining membrane and can be stabilized by cholera toxin incubation. Beta-cyclodextrin extraction of the membrane cholesterol removes the domains of reduced diffusion, but increases the interaction of GPI-anchored proteins with the membrane fences imposed by the membrane cytoskeleton. TrfR proteins only show interaction with the membrane fences.

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1520-Pos Board B290

Nucleated Pathways Involving Nanoparticles and Lipid Membranes

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The interactions of nanoscale objects at the membrane interface play an important role in cell biology, medical therapeutics, nanoparticle toxicity and antimicrobial peptides. In particular, these objects—through thermal fluctuations—can insert into membranes and subsequently undergo many interesting processes as nucleated (rare) events. To tackle the challenging nucleation problem, we need a method that can overcome the long time scales associated with these rare events without constraining the system to an artificially chosen reaction coordinate. Towards this end, we combine the string method with a dynamical version of the self-consistent field theory to compute the minimum free energy paths for nucleated events involving membrane-particle systems. We show that under tensions well below the metastability limit of the membrane, and in the regime where nucleation can occur on experimentally relevant time scales, there can be multiple pathways involving the particle: (1) particle-assisted membrane rupture, (2) particle translocation, and (3) membrane rupture from a metastable pore with an inserted particle.

1521-Pos Board B291

Molecular Dynamics Simulations of the Influenza Virus Envelope and Raft-Like Aggregates of Hemagglutinin

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The influenza virus envelope contains three transmembrane proteins (hemagglutinin, neuraminidase and M2) and is derived from the host cell membrane during viral assembly. The interactions of these proteins with their surrounding lipid environment are vital for many phases of the viral life cycle. In particular, their association with lipid rafts is thought to facilitate viral assembly, but many aspects of protein-raft interactions remain undetermined.

The envelope proteins have been simulated in domain-forming membranes at two different length scales, using the Martini coarse-grained force field. Aggregates of hemagglutinin were shown to associate with raft-type lipids in planar membranes, suggesting that high local protein concentration can be sufficient for association of transmembrane proteins with rafts in vivo. A realistic model of the entire viral envelope (84 nm diameter) was then built, comprising a lipid vesicle and inserted proteins. The 8 μs simulation represents one of the first MD studies of a complex biological membrane in near-atomistic detail, and may indicate why rafts do not form on larger length scales in vivo. The findings thus have important implications for our understanding of rafts and of the viral assembly process.